Apigenin isolated from *A. americana* encodes Human and *Aspergillus oryzae* S2 α -amylase inhibitions: credible approach for antifungal and antidiabetic therapies

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Abstract *Agave americana* extract was analyzed by reverse phase HPLC for characterization. Among phenolic compounds identified, apigenin was observed to be present. The finding showed an inhibitory effect of apigenin towards Human and *Aspergillus oryzae* S2 α -amylases. Apigenin inhibition towards Human and *A. oryzae* α -amylase activities was observed to be competitive. IC₅₀ and % inhibition of apigenin for *A. oryzae* α -amylase were 3.98 and 1.65 fold higher than for Human α -amylase. The inhibition of the described biocatalyst activity was significantly lowered when apigenin was pre-incubated with starch. In addition to the catalytic residues, 44 amino acid residues were involved on *A. oryzae* α -amylase-apigenin interactions while only 11 amino acid residues were exposed for Human α -amylase-apigenin complex. The binding site of apigenin showed 76 polar contacts for *A. oryzae* S2 α -amylase against 44 interactions for Human α -amylase. The docking studies confirmed the mode of action of apigenin and strongly suggested a higher inhibitory activity towards fungal amylase which was experimentally exhibited. These findings provided a rational reason to establish apigenin capability as a therapeutic target for postprandial hyperglycaemia modulation and antifungal therapy.

Keywords α -Amylase · *A. americana* · Apigenin · Competitive inhibition · Therapeutic potentiality and hyperglycemia · Antifungal therapy

Introduction

Agave was a perennial plant, distributed from arid and semi-arid regions like Mexico, and Africa. This plant had a high productivity of 10–34 Mg ha⁻¹ year⁻¹ in comparison to poplar wood (11 Mg ha⁻¹ year⁻¹) and switchgrass (15 Mg ha⁻¹ year⁻¹). *Agave* was a rich supply of bioactive composites and showed many biological activities, comprising anti-diabetic (Mellado-Mojica and López 2015), anti-bacterial, antioxidant capacity, and anti-inflammation potentialities. On the other hand, antihypertensive, immunomodulatory, anti-cancer, antiallergic and immunostimulation capability activities were also demonstrated (Moreno-Vilet et al. 2016). *Agave americana* was the most recognized among the 208 *Agave* species due to its potent biological activity and large availability. Phenolic compounds, including flavonoids, homoisoflavonoids and phenolic acids were found in several *Agave* species. Those phenols were considered as specific chemotaxonomic markers.

The worldwide occurrence of obesity has practically doubled in the last 25 years. Although initially linked with high income countries, it is now becoming widespread in low and middle-income nations. Certainly, by 2050 it may underlie 8 million cases (cumulative incidence) of heart disorders and 21 million cases of diabetes (Rtveladze et al. 2014). The identification of anti-diabetic bioactive compound could be useful in managing body weight and preventing overweight/obesity and their associated diseases. Several enzymes were used in starch hydrolysis to modify

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starch composition and attain desired functionality. Alpha amylases (endo-1,4- α -D-glucan glucanohydrolase EC 3.2.1.1) cleave α (1,4)-linkages between adjacent glucose units of starch polymers and other related compounds. The inhibition of mammalian alpha-amylases was considered as a therapeutic approach in diabetes and other related disorders (Miao et al. 2015). Traditional medicine plays a critical role in the treatment of various types of diseases. Indeed, natural products are effective and inexpensive and have fewer side effects.

Because of the requirement for more specific and less damaging antifungal therapies, and the emergence of resistant strains, the research of anti-enzyme molecules as a new antifungal approach was one of greatest attention. The dysfunction of the fungi α -amylase could cause several abiotic stresses and finally lead to the cell lyses. Indeed, glucose is not simply the biological fuel favored but also a signal molecule regulating gene transcription associated to glucose homeostasis and energy metabolism of fungi. Therefore, the glucose uptake inhibition by a dysfunction of microorganism α -amylase through natural compound could be a new safe approach for antifungal therapy. Some natural compounds demonstrated a set of promising biological activities comprising the both anti-microbial and antidiabetic effects such as polyphenols (Alqurashi et al. 2017). These polyphenols are low absorbed in the small intestine and they remain in the colon. In this latter, polyphenols modify the populations of human gut microbiota, the release of microbial metabolites, and the integrity of the intestinal and systemic metabolism (Martinez et al. 2017). Recent mechanistic works imply that these effects can be mediated, in part, by the polyphenols-dietary fiber bound (Marchesi et al. 2016). These polyphenols should be conjugated or associated to sugars, sugar alcohols or amines and dietary fiber that can be liberated through gastrointestinal digestion.

Apigenin (4',5,7-trihydroxyflavone), belongs to the flavone group which is a class of flavonoids based on the backbone of 2-phenylchromen-4-one (2-phenyl-1-benzopyran-4-one). Apigenin was found in a wide variety of edible plants comprising parsley, oranges, onions, tea, wheat sprouts, and chamomile. The in vitro experiments and animal studies, showed a multiplicity of prospective biological activities. Apigenin had a wide spectrum of biological activities like antioxidant, anti-inflammatory, antitumor, and neuroprotective agent (Yan et al. 2014). Additionally, it exerted an inhibiting activity. Indeed, it was found as xanthine oxidase inhibitors in a competitive type (Su et al. 2015) and exhibited a mixed-type inhibition on Human cytochrome (Kimura et al. 2010). Apigenin beneficial medicinal effects may be due to its abilities to inhibit calcium influx, improve vasodilation,

vasorelaxation, scavenge oxygen free radicals, and counteract cell death (Preedy 2013).

Compared with the synthetic drugs, the natural molecules screened from a multitude of plants have become more appropriate anti-diabetic agents (Miao et al. 2015; Lo Piparo et al. 2008). Apigenin is also described as an antidiabetic agent using in vivo test. However, the mechanism about the anti-diabetic process remains elusive. Our study was involved in the characterization of one flavone compound from *A. americana* leaves namely apigenin. We also investigated its inhibitory effects on Human and *A. oryzae* S2 α -amylase activities and its molecular process.

Materials and methods

Biological materials

The leaves of *A. americana* were collected from Jelma state (the town of Sidi Bouzid-Tunisia).

Chemical materials

Human pancreatic α -amylase, apigenin, acarbose, ethyl acetate, starch from potato, acetonitrile and 3, 5-dinitrosalicylic acid were purchased from Sigma-Aldrich (St. Louis, MO, USA). Apigenin was dissolved in DMSO before use. *A. oryzae* S2 amylase (AmyA) was purified as described in a previous work (Sahnoun et al. 2011), and used to test the effect of apigenin towards fungi amylases.

Preparation of *Agave americana* extract

Leaves of *A. americana* were washed, freeze dried, harvested into a juice form, mixed with ethyl acetate, sonicated (20 min) (Sonicator FS30HFisher Scientific, USA), centrifuged (28,620 $\times g$ /30 min/20 °C) and the supernatant was recovered. The solvent was discarded under vacuum (40 °C) with a rotary evaporator (BÜCHI Labortechnik AG, Switzerland) and the dried *A. americana* extract was stored at 4 °C until its use. Briefly *A. americana* extract was dissolved in DMSO at different concentrations (10, 50, 100, 200, 300, and 400 $\mu\text{g/mL}$).

Effect of *Agave americana* extract and apigenin on the Human and *A. oryzae* S2 α -amylase activities

The α -amylase inhibition assay was determined with potato starch as substrate. Potato starch solution [1% (W/V)] was dissolved in phosphate buffer (100 mL, 0.2 M, pH 5.2) and gelatinized for 20 min at 80 °C. 100 μL of the above *A. americana* extract or apigenin was incubated with 350 μL phosphate buffer (100 mL, 0.2 M, pH 5.2) and 50 μL of α -

amylase (100 U/mL) in phosphate buffer (100 mL, 0.2 M, pH 5.2). The tubes were incubated in a water bath at 37 °C for 20 min, and added with 500 µL 1% (W/V) potato starch solution. The reaction mixture was incubated at 37 °C for 30 min and then the reaction was stopped with 3 mL of dinitrosalicylic acid reagent. Thereafter, the mixture was boiled for 10 min and cooled to room temperature. Next, the reaction mixture was diluted by adding 10 mL of deionized water and the absorbance was measured at 550 nm. The α -amylase activity was determined as U/mL, where the unit was the amount of enzyme used to release one µmol of glucose equivalent per minute under the assay conditions.

Denatured enzyme solution was obtained by boiling native enzyme in a water bath for 15 min. Percentage of apigenin inhibition towards α -amylase was calculated according to the equation below:

$$\% \text{ Inhibition} = \frac{(A_{\text{control}} - A_{\text{control blank}}) - (A_{\text{sample}} - A_{\text{sample blank}})}{(A_{\text{control}} - A_{\text{control blank}})} \times 100.$$

Where the denatured enzyme was used as blank test. The control test was done in absence of inhibitor by replacing it by DMSO at the same amount. The sample test contained native enzyme and inhibitor. IC₅₀ value (half maximal inhibitory concentration) was obtained graphically by an inhibition curve.

Influence of apigenin and *Agave americana* extract concentrations

The checking of reversibility was done by measuring enzyme activity in the presence of ligand and comparing this value with the one obtained after extensive dialysis of the enzyme–ligand solution. The residual α -amylase activity in the presence of *A. americana* extract at different concentrations (10, 50, 100, 200, 300, and 400 µg/mL) was done. The detection of the inhibition mode of apigenin towards Human and *A. oryzae* S2 α -amylases was carried out with increasing concentrations of potato starch as a substrate [0.5, 1, 1.5, and 2% (W/V)]. The catalysis kinetics values of α -amylases in the presence of apigenin at different concentrations (2, 30, and 60 µM) were determined. 100 µL of apigenin solution and 50 µL of α -amylase solution (100 U/mL) were mixed first and incubated at 37 °C for 20 min, followed by the addition of 500 µL of different concentrations of potato starch solution for 30 min at 37 °C. The type of inhibition was determined by Lineweaver–Burk (LB) plot analysis of the data, which was calculated from the results according to Michaelis–Menten kinetics model: $V = V_{\text{max}}[S]/K_m + [S]$.

Influence of pre-incubation of apigenin with potato starch solution

The effect of incubation order on α -amylase activity was evaluated using potato starch as substrate with the same method as described above, except that 100 µL of apigenin (2, 30, and 60 µM) and 500 µL of potato starch solution were kept first at 37 °C for 20 min. Then we add 50 µL of α -amylase solution and 350 µL of phosphate buffer.

Biochemical analyzes meaning HPLC technology

Agave americana (L.) extract was submitted to a reverse phase HPLC–DAD (Agilent, Series 1260, Waldbronn, Germany). The instrument comprises an online degasser, a quaternary pump, an auto sampler and a thermostatically controlled column compartment. The separation was achieved on a ZORBAX Eclipse XDB-C18 column serial number USNH027266 (4.6 mm I.D. $\times$ 250 mm $\times$ 3.5 µm particle size) at a flow rate of 0.5 mL/min. The operating temperature was fixed at 40 °C. The volume Fractions were eluted from the column with two mobile phases, namely A (100% water) and B (100% acetonitrile), as follows: from 0 to 10 min (80% A, 20% B), from 10 to 30 min (50% A, 50% B), from 30 to 40 min (100% B), from 40 to 50 min (50% A, 50% B) and from 50 to 60 min (80% A, 20% B). The fractions were monitored at 280 nm. Apigenin (Sigma) was used as standard with a limit of quantification and detection of 0.023 and 0.0049 µg/mL respectively.

Docking studies

The three-dimensional structure of Human pancreatic α -amylase was imported from the Protein Data Bank (1HNY). The 3D structural model of the AmyA was generated using the SWISS-MODEL (<http://www.expasy.org/swissmod/>) and the crystal structure of α -amylase from *A. niger* (PDB accession code 2GUY_A) as template which possesses 99% sequence identity with AmyA (Sahnoun et al. 2016). Docking was performed with Autodock vina. Grid box of 40 $\times$ 40 $\times$ 40 points and 70 $\times$ 20 $\times$ 70 was used for Human and AmyA amylases respectively with a spacing of 1.0 Å. The grid box center was put on $x = 12.33$, $y = 57.47$, and $z = 21.2$, and $x = 9.32$, $y = 16.1$, and $z = 4.31$ for Human and AmyA respectively. The gasteiger charges were assigned to protein and ligand molecules. The exhaustiveness was set on 20. The best pose for the ligand was obtained. An extra blind docking was also achieved with the use of the Swissdock web server (<http://swissdock.vital-it.ch/>).

Statistical analyses

All the experiments were achieved in triplicate. The results are presented as $x \pm \text{SD}$, where x refers to the mean of at least three replications and SD to the standard deviation. Student's t test was used to determine the significance of differences between means. Significant difference was considered at $p < 0.05$. The data were analyzed by SPSS for windows (Version 11.0.1, 2001, LEAD Technologies, Inc., USA).

Results and discussion

The behavior of the α -amylases catalytic assessment in presence of the *A. americana* extract

The results showed that the *A. americana* extract increased the α -amylase activities of both Human and *Aspergillus* origins. This activation was reversible since it was removed after extensive dialysis of the enzyme-*Agave americana* extract. We noted that this activation also persisted after *A. americana* extract heat treatment (100 °C, 10 min). This result suggested the presence of a none proteinaceous activation effector. It could be an anionic activation since ions could increase α -amylase activity. However, this activation was maintained after EDTA chelation of *A. americana* extract. By another way, it was reported that phenolic compounds might enhance α -amylase activity (Kashani-Amin et al. 2013). The effect of *A. americana*

extract activation was improved at low concentrations, progressively reached a peak, then gradually decreased at high concentrations. This effect was well linked with the effect of some polyphenols which enhanced amylase activity at low concentrations and inhibited it at high concentrations (Yang and Kong 2016). Hence, the activation effect from *A. americana* is likely to be due to its containing polyphenols.

Peculiar role of apigenin

The fractionation of the *A. americana* extract on reverse phase HPLC seeking for polyphenols is illustrated in Fig. 1. Among these, apigenin with a lower content (10%) was identified. The last one was founded in several fruit juice, tea and wine as a major phenolic compound. People have consumed juice, tea and wine for thousands of years, holding the belief that these drinks can help digestion. Indeed, Liu et al. (2011) has shown that polyphenols enhance the regulation and the rate of the digestive system and can be considered as digestion aid. Hence, even though the global role of phenolic compound on digestive enzymes was well addressed, the specific effect of each composite was not well established. Hereafter, the effect of apigenin on Human α -amylase activity was studied. We have also tested the effect of apigenin towards digestive fungal amylase namely *A. oryzae* α -amylase in order to enlarge this concept for fungi metabolism and for the research of anti-fungzyme molecules as a new antifungal approach.

Fig. 1 HPLC Chromatogram of *Agave americana* extract (a), and apigenin (b)

Fig. 2 Determination of the type of inhibition observed with apigenin towards *A. oryzae* S2 amylase (a) and Human pancreatic α -amylase (b). Reverse values of activity (V) and substrate concentration ([S]) were plotted in the absence of apigenin (filled diamond),

and the presence of three different concentrations of the compound: 2 μ M (filled square), 30 μ M (filled triangle), 60 μ M (filled circle)

Apigenin: good alternative for Human pancreatic and *A. oryzae* α -amylase activity modulations

An inhibitory effect towards Human pancreatic and *A. oryzae* α -amylase was observed for apigenin (both for the standards solution and the collected pic of HPLC from the *A. americana* extract). This inhibition was reversible since it was removed after an extensive enzyme-apigenin dialysis. From the double-reciprocal (Lineweaver–Burk) plots between $1/[S]$ (starch concentration (mg/mL)) and $1/V$ (reaction rate (U/mL)) (Fig. 2a, b), apigenin inhibition mode towards Human and *A. oryzae* α -amylase activities belonged to the competitive one. In a previous study on Human pancreatic α -amylase, myricetin, and trans-chalcone were also classified to be a competitive inhibitor of mammalian α -amylase (Najafian et al. 2011). In the case of luteolin and genistein, mixed inhibition behavior was attributed towards porcine α -amylase with the use of a chromogenic maltoheptaoside as a substrate (Tadera et al. 2006). Since there are still few works on the characterization of the phenolic compounds inhibition against α -amylase, a clear conclusion cannot be formulated. Compared to flavonoids, apigenin could be a smaller compound able to find the catalytic crevice of the enzyme's surface. For 30 and 60 μ M the apigenin inhibitory activity was

increased significantly ($p < 0.05$) for both amylases regarding all the concentration interval of substrates (5–20 mg/mL) used. Nevertheless, in presence of 2 μ M apigenin concentration this inhibition was significantly ($p < 0.05$) detected only for low starch concentration (5, and 10 mg/mL). We can conclude in this case that at high substrate concentration (10–20 mg/mL) the interaction of starch-apigenin could dominate the enzyme-apigenin interaction.

Dixon plots were subsequently drawn for apigenin (Fig. 3a, b). The K_i were 29 μ M and 3.9 μ M for Human pancreatic and *A. oryzae* α -amylases, respectively. The IC_{50} and the % inhibition of the apigenin for *A. oryzae* α -amylase were 3.98 and 1.65 fold higher than for Human α -amylase (Table 1). It was observed that, apigenin (Figs. 2, 3) was more effective ($p < 0.05$) in inhibiting the α -amylase originating from fungi than for the human. In fact, apigenin have more affinity and selectivity for fungal amylases than that for the human. This effect could be attributed to the differences in size and location of apigenin binding pocket towards these two amylase types. These in vitro findings also revealed that apigenin was a potent strong reversible inhibitor of α -amylases and was more efficient in comparison to other natural inhibitors already described (Table 1).

Fig. 3 Dixon plot was used to calculate the K_i of apigenin towards *A. oryzae* S2 amylase (a) and Human pancreatic α -amylase (b). Reverse values of activities (V) were plotted in the presence of inhibitor ([I]) at different concentration values, with the use of four different

concentrations of the substrate expressed as (mg/mL): 5 (filled diamond), 10 (filled square), 12.5 (filled triangle), and 20 (filled circle)

Indeed, comparing to several studied anthocyanidin compounds which belong to flavonoids inhibitors class, a lower inhibition, varying to 12–21% was detected with a maximum inhibitory concentration of 3.87 mM (Worsztynowicz et al. 2014). With respect to hydroxycinnamic acid derivatives, like trans-cinnamic, chlorogenic acid, isochlorogenic acid, and neochlorogenic acid, a lower inhibitor efficiency was revealed with a maximum of 50% inhibition activity at 0.6–0.8 mM for isochlorogenic acid (Worsztynowicz et al. 2014; Funke and Melzing 2006; Chethan et al. 2008). However, based on IC_{50} value, flavonoids like quercetagenin, fisetin, quercetin had a higher inhibition activity for salivary α -amylase and porcine pancreatic α -amylase (Manaharan et al. 2012). This suggests that the steric position of the hydroxyl groups and the oside incorporation in the backbone of flavonoids are a key factor of the inhibition strength.

Apigenin: potential for therapeutic use for hyperglycaemia and antifungal agents

Postprandial hyperglycaemia is the earliest metabolic aberration to occur in Type-2 diabetes mellitus. Among powerful Human α -amylase inhibitor, the acarbose was considered as a postprandial hyperglycaemia drug and taken in the clinical management of early diabetes. Apigenin exhibited an IC_{50} of 75.12 μM was a 1.74-fold of

that of acarbose (acarbose, $IC_{50} = 43$ μM). Hence, apigenin could be considered as a natural therapeutic target for modulation of postprandial hyperglycaemia.

The influence of incubation order of apigenin and starch with α -amylase on the inhibitory activity was studied. The enzyme inhibitory activity was significantly lowered (inferior than 15% at 5 mg/mL substrate) when apigenin was pre-incubated with starch before the α -amylase addition. The study revealed that apigenin could not only bind and inhibit α -amylase but also bind to starch hence, disturbing its inhibitory efficiency towards the enzyme. Indeed, Liu et al. (2011), and Xiao et al. (2013) reported that the phenolic compounds interacted with starch by hydrogen bonds. This result suggests that drinking juice, after a meal is more valuable for the starch digestion than before a meal, and is consistent with the belief that drinking juice after a meal helps digestion.

The increasing resistance against available antifungal agents is of main worry. There is also a concern regarding the rising pathogenicity of the less virulent species. New antifungal compounds research was therefore needed. The physiological and biochemical similarities among human and fungal cells provide many issues to think for substances with harmful actions and specific antifungal actions. Additionally, some flavonoids are recently recognized as antimicrobial barriers (Qing-Hu et al. 2015). From these current works, the response mechanism of the

Table 1 Inhibitory activity of plant compounds

Compound	Source	Inhibitory activity	References
Proanthocyanidins			
Epicatechin	Green tea extract	63.5%, IC ₅₀ = 2.07 mg/mL	Miao et al. (2015)
Epigallocatechin gallate			
Epicatechin gallate			
Tannin			
Phlorotannin	Ascophyllum nodosum	49% at 3 µg/mL	Roy et al. (2011)
Flavonoid			
Apigenin	Agave americana	Human α-amylase: K _i = 29 µM, IC ₅₀ = 75.12 µM, 45.83% at 60 µM A. oryzae S2 α-amylase: K _i = 3.9 µM, IC ₅₀ = 18.83 µM, 76.03% at 60 µM	This study
Hydroxycinnamic acid and Coumarin derivatives			
Trans-cinnamic	Finger millet malt	K _i ' = 7.30 × 10 ⁻⁷ M, K _i = 66.7 µM mixed non-competitive	Chethan et al. (2008)
Chlorogenic acid		50% at 1.4–1.6 mM	Funke and Melzing (2006)
Isochlorogenic acid		50% at 0.6–0.8 mM	
Esculin		50% at 1.4–1.6 mM	
Neochlorogenic acid	Aronia melanocarpa	44.25%, IC ₅₀ = 2.58 mg/mL	Worsztynowicz et al. (2014)
Anthocvaniduis			
Cyanidin-3-galactoside	Aroma melanocarpa	12.18% at 5.16 mg/mL	Worsztynowicz et al. (2014)
Cyanidin-3-glucoside		13.56% at 1.74 mg/mL	
Cyanidin-3-arabinoside		21.46% at 3.98 mg/mL	
Cyanidin-3-xyloside		13.76%	
Hydroxybenzoic acids			
Gallic acid	Finger millet malt	K _m = (0.625%) mixed non-competitive inhibition	Chethan et al. (2008)
Vanillic acid			
Terpenes			
Squalene	Centratherm arthelinticwn	30%	
Oleanolic acid		± 55%	
Caffeicacid		50% at 185 µg	

microbial infection towards flavonoids remained unknown and some researchers suggested that flavonoids might act on the electron transport chain, inducing the lysis of mycelium (Ilboudo et al. 2016). Apigenin showed a strong anti-enzymatic activity of *A. oryzae* α -amylase. It could therefore, be used as strong antifungal agent. Since there are no works on the characterization of phenolic inhibitors compounds against α -amylase from fungi, a clear comparison cannot be formulated. Based on this fact and our findings, we will look for the plausible opportunity to formulate a novel approach using those molecules as antifungal molecules in which fungizymes are the host.

Docking studies

The GH13 α -amylases are three-domain proteins. The domain A is the main catalytic (β/α)₈-barrel domain. The domain B is a small domain connecting the strand β ₃ to the third helix α ₃ structuring a substrate binding cleft at the interface of domains A and B. The domain C is formed by an antiparallel β -sheet linked to domain A. The catalytic mechanism, consisting of an aspartic acid as catalytic nucleophile in strand β ₄, a glutamic acid as proton donor in strand β ₅, and an aspartic acid as transition-state stabilizer in strand β ₇. It is known that Human pancreatic α -amylase

have a three conservative domains as domain A (residues 1–99 and 169–404) domain B (residues 100–168) and domain C (residues 405–496) and have Asp197, Glu233, and Asp300 as a three putative active site residues. The three conserved domains of *A. oryzae* α -amylase were known as follow domain A (residues 1–122 and 181–383) domain B (residues 123–180) and domain C (residues 384–476). The catalytic *A. oryzae* catalytic triad consisted of Asp 206, Glu230, and Asp 297 (Sahnoun et al. 2016).

A docking experiment was done on the whole structure of the Human and *A. oryzae* S2 α -amylases, and the main docking poses were obtained respectively in the active site (Fig. 4a, b). As shown in Fig. 4b, the apigenin was surrounded by 11 amino acid residues of the Human α -amylase, including Trp58, Trp59, Trp62, Gln63, His 101, Leu156, Leu162, Thr163, His299, Ala198, Arg195. Since apigenin interacted with the three catalytic residues (Asp197, Glu233, and Asp 300), it could be concluded that it acts with a competitive inhibitory mode. Whereas the apigenin was surrounded by the three catalytic residues (Asp 206, Glu230, and Asp 297) of *A. oryzae* α -amylase and 44 amino acid residues comprising Phe13, Leu15, Thr16, Arg18, Thr37, Trp61, Ile62, Thr63, Pro64, Thr66, Gln68, Leu69, Tyr75, Ala78, Tyr79, His80, Gly81, Tyr82, Trp83, Gln84, Tyr94, Met115, Val118, Val119, His122, Met123, Trp165, Leu166, Asp168, Val171, Leu173, Pro174, Leu203, Arg204, Ile205, Thr207, Ile228, His296, Tyr328, Asp340, Pro341, Arg344. Therefore, apigenin also

acts as competitive inhibitor for *A. oryzae* S2 α -amylase. The in silico study confirms the mode of action of apigenin against these two α -amylases already revealed by the above in vitro studies. The aminoacids comprising His80 and Asp340 which contributing to the interaction apigenin-*A. oryzae* S2 α -amylase were also involved in the maltotriose-AmyB complex interaction. Tyr75, Arg204, Pro341, Arg344 were also interfering especially in the maltotriose-AmyA complex interaction. Where, AmyA and AmyB were a two extracellular amylases from *A. oryzae* S2 (Sahnoun et al. 2011), and maltotriose is a frequently-used in the ligand- α -amylase interaction studies (Housaindokht et al. 2013). A more precious in silico study concerning the revelation of the number, the category, the distance (Å), and type of the interactions displayed by apigenin towards the both α -amylases is resumed in Table 2. It was clearly seen that the binding site of apigenin concerning *A. oryzae* S2 α -amylase exposed more polar contact number (76 interactions) with this ligand than that of Human α -amylase (44 interactions). The docking studies strongly suggested a higher inhibitory activity of apigenin towards fungi than Human amylase as it exhibited in vitro.

It should be noted that the interaction with the catalytic residues of Human α -amylase for flavonoids, was also described in silico (Lo Piparo et al. 2008). The lack of this interaction was linked to a lower inhibitory power (Lo Piparo et al. 2008). In addition, flavonoids-Human salivary α -amylase interaction were located in the active site with

Fig. 4 Details of the interaction between *A. oryzae* S2 amylase (a) and Human pancreatic α -amylase (b) and apigenin. The implicated catalytic residues were labeled by red

Table 2 *In silico* study results of the apigenin inhibitory effect towards *A. oryzae* S2 and Human α -amylases

color	Distance (Å)	Category	Types	Number of interaction	
				Apigenin towards <i>A. oryzae</i> S2 α -amylase	Apigenin towards Human α -amylase
	> 3	Electrostatic	Salt Bridge	3	1
	> 3.4	Electrostatic	Attractive Charge	4	0
	3.21	Electrostatic	Pi-Cation	1	0
	3.85	Electrostatic	Pi-Anion	1	0
	4.04	Electrostatic	Pi-Anion	1	0
	2.77886	Hydrogen Bond	Conventional Hydrogen Bond	0	1
	>2.8	Hydrogen Bond	Water Mediated Hydrogen Bond	0	5
	>2.7	Hydrogen Bond	Water Hydrogen Bond	0	12
	>1.7	Hydrogen Bond	Conventional Hydrogen Bond	29	8
	>3.1	Hydrogen Bond	Carbon Hydrogen Bond	9	4
	>3.8	Hydrogen Bond	Pi-Donor Hydrogen Bond	0	2
	2.78741	Other	Pi-Lone Pair	0	1
	>3.69647	Hydrophobic	Pi-Sigma	4	0
	>4.5	Hydrophobic	Pi-Pi Stacked	3	1
	>4.3	Hydrophobic	Pi-Pi T-shaped	6	2
	4.2069	Hydrophobic	Amide-Pi Stacked	1	0
	>4.5	Hydrophobic	Alkyl	6	0
	>3.89136	Hydrophobic	Pi-Alkyl	8	7
Total interaction				76	44

exhibition of a π - π interaction of the aromatic components of flavonoids among Trp59 (Najafian et al. 2011). Furthermore, Trp59 interaction was marked to be a regular event in the case of flavonoids docking concerning the porcine pancreatic enzyme, whereas Tyr62 was implicated in some cases (Lo Piparo et al. 2008). Ramasubbu et al. (2004) also highlighted the Trp59 as a key residue involved in hydrogen bonds and stacking with the substrate. It could be obvious that an interaction of inhibitors with these residues would be certainly relevant in their effectiveness.

Conclusion

This study showed that *A. americana* ethyl extract improved the amylase activity by a reversible activation. This activation is more important at low concentration,

gradually reached a peak, and then progressively lowered at high extract dose. The activity measurement showed that the isolated apigenin (10%) exhibited a competitive inhibition towards human and *A. oryzae* α -amylase. The activation effect of *A. americana* extract on α -amylase activity may be related to others phenolic compounds that counteract the mild inhibition effect of the contained apigenin. Through those findings, we seek to establishing a post-prandial hyperglycemia modulation therapy. Based on our findings, we will also look for the plausible opportunity to formulate or to think abode a novel approach using those molecules and other like ones as antifungal drugs in which fungizymes are the basic host.

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Compliance with ethical standards

Conflict of interest None declared.

Human and animals rights This article does not contain any studies with Human participants or animals performed by any of the authors.

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